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(54) BLOOD FILTER FILM AND METHOD THEREFOR

(57)Abstract:
PROBLEM TO BE SOLVED: To provide a method for acquiring blood serum, blood plasma, blood corpuscles, or leukocytes from a small amount of whole blood.
SOLUTION: This blood filter film is made of a film of hole diameters between 0.5-40 μm and of the coefficient of variation of dispersions of the hole diameters is 20% or less, and this blood filter method uses two such films or more of the same average hole diameters or different average hole diameters.

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* NOTICES *

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CLAIMS

[Claim(s)]
[Claim 1] Hemofiltration film with which the variation in an aperture consists [an aperture] of film of 20% or less of coefficient of variation by 0.5-40 micrometers [claim 2] Hemofiltration film according to claim 1 which has honeycomb Mr. structure [claim 3] Hemofiltration film according to claim 1 or 2 which consists of matter which is mainly concerned with a Polly epsilon-caprolactone [claim 4] The hemofiltration approach by using the film of the same average aperture according to claim 1 to 3, or two or more different film of an average aperture

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* NOTICES *

DETAILED DESCRIPTION

[Detailed Description of the Invention]
[0001]
[Field of the Invention] This invention relates to the filtration membrane for removing a corpuscle from whole blood, and the method of catching and collecting an erythrocyte or leucocytes from whole blood using the filtration membrane.
[0002]
[Description of the Prior Art] Measurement of classes, such as the constituent in blood, for example, metabolite, protein, a lipid, an electrolyte, an enzyme, an antigen, and an antibody, or concentration is performed considering the blood serum or plasma obtained by usually carrying out centrifugal separation of the whole blood as a specimen. However, the centrifuge method which needs a centrifugal separator by making the electrical and electric equipment into power is unsuitable for floor inspection for centrifugal separation to take time and effort and time amount, and process especially a small number of specimen in a hurry. Then, the approach of carrying out separation recovery of a blood serum or the plasma from whole blood by filtration has been examined.
[0003] The electrode holder made from plastics is loaded with the glass fiber filter paper of 3-6 sheets, and there is a method of filtering whole blood by suction among the approaches of obtaining a blood serum or plasma from whole blood by filtration as it is marketed as "a plasma filter PF" by Fuji Photo Film Co., Ltd. and is known for JP,2000-81432,A. However, by this filtration approach, although plasma can be filtered about [150micro] L, whole blood cannot be 3mL extent needed, and whole blood of a minute amount cannot be filtered.
[0004] A corpuscle is caught from whole blood, there is the **** approach among the approaches of obtaining a blood serum or plasma, about the three-dimension porous body indicated by JP,10-185910,A, and the polysulfone film, the cellulose acetate film, etc. are known by filtration. However, it is difficult for this film to create an aperture to homogeneity.
[0005] The technique of catching and extracting a leucocyte from whole blood, collecting genes based on this on the other hand, and using for a gene analysis or gene diagnosis being performed briskly in recent years, and collecting leucocytes from whole blood is needed.
[0006] The polyurethane porosity filter put in practical use in "immuno guard (trademark) III-RC" by TERUMO [CORP.] CORP., the polyester filter put in practical use in "pole transfusion filter PL1J" currently sold at Japanese Pole company are known by the approach of removing only a leucocyte from whole blood. However, this filter is for removing a leucocyte from the whole blood for transfusion, and it is difficult to catch a leucocyte from the whole blood of a minute amount, and to collect leucocytes.
[0007]
[Problem(s) to be Solved by the Invention] When only the whole blood of minute amounts, such as a newborn infant, can collect blood, the method of obtaining a blood serum or plasma from the whole blood of a minute amount in a hurry is searched for. Moreover, in order to use for gene diagnosis etc. in the approach of catching a corpuscle from whole blood by filtration, the method of carrying out fractionation only of the leucocyte and extracting it is searched for.
[0008]

[Means for Solving the Problem] It found out that only a corpuscle can be caught from the whole blood of a minute amount, and a blood serum or plasma can be collected by using the thin film of a uniform aperture, and that only the leucocytes of whole blood could be caught and collected with the magnitude of a uniform aperture.
[0009]
[The mode of implementation of invention] Although the high molecular compound which dissolves in nonaqueous solubility solvents, such as a Polly epsilon-caprolactone, Polly 3-hydroxy butyrate, agarose, Polly 2-hydroxyethyl acrylate, and polysulfone, can be used as a membranous material, it is desirable to use a Polly epsilon-caprolactone.
[0010] Although at least these materials can make the film of the honeycomb's structure form, it is desirable to add an amphiphilic material. As an amphiphilic material, there is amphiphilic polyacrylamide, for example.
[0011] the mixing ratio of a membranous material and an amphiphilic material -- as for a rate, it is desirable to use it in the range of the weight ratios 0:1-1:0. It is the range of the weight ratios 5:1-20:1 more preferably.
[0012] What is necessary is just the solvent of nonaqueous solubility in which the high molecular compound used as membranous materials, such as chloroform, dichloromethane, a carbon tetrachloride, and a cyclohexane, can be dissolved as a solvent which carries out the cast. In order to mass-produce industrially, as for the polymer concentration when carrying out the cast, it is [that what is necessary is just the concentration which can form a poly membrane] desirable to produce a film by the highest possible concentration beyond 0.1wt%.
[0013] Since the material of the film of nonaqueous solubility, for example, a Polly epsilon-caprolactone, is dissolved in the solvent of nonaqueous solubility, for example, chloroform, when evaporating a solvent with high humidity air, the moisture in air dews with heat of vaporization, and with evaporation of a solvent, it grows gradually and becomes the waterdrop of size with a diameter of about 0.5-40 micrometers. Since the Polly epsilon-caprolactone of nonaqueous solubility cannot be dissolved in this waterdrop, the film with which this part became a hole (pore) is obtained. For example, since the cast is carried out to the petri dish two-dimensional, grown-up waterdrop is regularly arranged to the two-dimensional close packed structure of a ball, and the film of honeycomb structure is obtained as a result.
[0014] An aperture is controllable by adjusting the concentration and volume of liquid which carry out the cast, supplying supporters, such as a petri dish, and controlling the evaporation speed of a solvent, and dew condensation speed by controlling the temperature of an ambient atmosphere or the air to spray, and humidity. Furthermore, the film of more regular honeycomb structure can be created by adding the surfactant of a minute amount, and suppressing and stabilizing fusion of waterdrop.
[0015] Although the relative humidity of 30% and 80% of thing were mainly used for the high humidity air sprayed on the film, that what is necessary is just the humidity which the moisture in air can be made to dew on the surface of the film, with temperature, it should just be 20 - 100% of relative humidity, and comparatively inactive gas, such as not only air but nitrogen, an argon, etc., may be used for it.
[0016] The flow rate of the high humidity air sprayed on the film should just be a flow rate which can evaporate the solvent which the moisture in air could be made to dew on the surface of the film, and was used for the cast.
[0017] As for the temperature of the ambient atmosphere when spraying high humidity air, on laboratory level, it is [that what is necessary is just the temperature to which the solvent used for the cast can evaporate] desirable that it is the temperature of 5-80 degrees C on 15-32 degrees C and production level.
[0018] By changing the relative humidity, the temperature, and the flow rate of the concentration of the solution which carries out the cast, the amount of a solution, the class of solvent, and the air to spray Dew condensation, growth of waterdrop, and the vapor rate of a solvent can be controlled, and the film of the structure of the regular honeycomb of various apertures can be obtained. It can be used as a filter which can separate each by changing the size of an aperture for the erythrocyte which is the diameter of about 7 micrometers although the platelet with a

diameter of about 3 micrometers which is a component in blood, a leucocyte with a diameter of about 15 micrometers, and deformability are large.

[0019] Moreover, capacity separable as a filter can be heightened by carrying out the laminating of the almost equal film of an aperture. Furthermore, by carrying out the laminating of two or more film with which apertures differ, or it divides some biological substances into coincidence, fractionation can be carried out. For example, catching a leucocyte by the large film of an aperture and catching an erythrocyte by the small film of an aperture can attain to coincidence by carrying out the laminating of the film of 5.5-8.5 micrometers of apertures, and the film of 3.5 micrometers or less of apertures, and supplying whole blood from the large film side of an aperture.

[0020] Furthermore, expectation as alternative separation collection management technique of a target biological substance which is represented with setting an aperture as submicron order by blood purification, such as dialysis, can be performed.

[0021] In this invention, two or more holes with an almost fixed aperture arrange honeycomb Mr. structure regularly, and it means the structure where such a hole has penetrated the film. There is especially no limitation in the cross section of a hole, and it is good for it in configurations, such as circular, an ellipse form, a hexagon, a rectangle, and a square.

[0022]

[Example] Hereafter, although an example explains this invention still more concretely, the range of this invention is not limited to the following example.

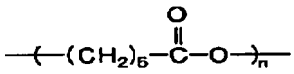
[0023] Example 1 Creation of the film of honeycomb Mr. structure (1)

The chloroform solution (0.1 - 2wt% as polymer concentration) which mixed the Polly epsilon-caprolactone (compound 1) and amphiphilic polyacrylamide (compound 2) of average molecular weight 70,000-100,000 at a rate of 10:1 by the weight ratio By carrying out 5mL cast, spraying the high humidity air of 30 - 80% of relative humidity by the flow rate of per minute one to 20 L on a petri dish with a diameter of 10cm, and evaporating a chloroform solvent, it has flexibility and elasticity and the film of the honeycomb Mr. structure where dynamics reinforcement is strong was obtained.

[0024]

[Formula 1]

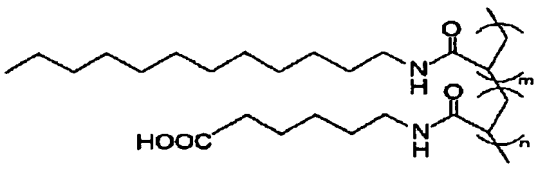
化合物1



[0025]

[Formula 2]

化合物2



(m:n=4:1)

[0026] When the structure of the film created on the above-mentioned various conditions was observed with the optical microscope and the scanning electron microscope, it was the film of the structure of the honeycomb of a 0.5-40-micrometer aperture, and was the structure penetrated from the front face with the hole of a monolayer to the rear face. The aperture was carrying out the beautiful round shape over the whole surface which carried out the cast, and its size was also almost the same. When the aperture was measured from the taken photograph and distribution of an aperture was searched for, it turned out with coefficient of variation that it is less than [valve flow coefficient10%]. Moreover, since the 10th more than diffracted light was

observed over the whole film surface which carried out the cast by evaluation of light scattering using laser, it turned out that the very high porous film of regularity was able to be created.

[0027] Example 2 Creation of the film of honeycomb Mr. structure (2)

Solvent dependence of the film which can be created when changed the solvent which carries out the cast with chloroform, benzene, toluene, and a xylene using the amphiphilic polyacrylamide which consists of a compound 2 as matter used as the film, solution concentration was set to 1.0g/L, set the amount of casts to 30microL, use glass for the substrate which carries out the cast, the flow rate of high humidity air is made a part for 0.09L/, relative humidity is made 85% and temperature is made into 20 degrees C was evaluated. The gestalt observation result at this time is shown in drawing 1 . In drawing 1 , an aperture is 0.5 micrometers - 10 micrometers from a top.

[0028] Example 3 Creation of the film of honeycomb Mr. structure (3)

The amphiphilic polyacrylamide which consists of a Polly epsilon-caprolactone which consists of a compound 1 as matter used as the film, and a compound 2 is used at a rate of 10:1 by the weight ratio. The amount which carries out and carries out the cast of the solution concentration to 10.0 g/L is changed with 5, 10, and 20mL, using chloroform as a solvent. The amount dependence of casts of the film which can be created when use glass for the substrate which carries out the cast, the flow rate of high humidity air is made a part for 2.0L/, relative humidity is made 30% and temperature is made into 20 degrees C was evaluated. The gestalt observation result at this time is shown in drawing 2 . In drawing 2 , an aperture is 8 micrometers - 35 micrometers from a top.

[0029] Creation of the film of example 4 honeycomb Mr. structure (4)

The amphiphilic polyacrylamide which consists of a Polly epsilon-caprolactone which consists of a compound 1 as matter used as the film, and a compound 2 is used at a rate of 10:1 by the weight ratio. Solution concentration is changed with 1, 5, 10, and 20 g/L, using chloroform as a solvent. The amount of casts was set to 10mL(s), and solution concentration dependence of the film which can be created when use hydro gel for the substrate which carries out the cast, the flow rate of high humidity air is made a part for 2.0L/, relative humidity is made 30% and temperature is made into 20 degrees C was evaluated. The gestalt observation result at this time is shown in drawing 3 . In drawing 3 , apertures are a minimum of 15 micrometers and a maximum of 25 micrometers.

[0030] Example 5 Creation of the film of honeycomb Mr. structure (5)

The Polly epsilon-caprolactone which consists of a compound 1 as matter used as the film is used. Using chloroform as a solvent, make solution concentration into 1.0 g/L and the amount of casts is set to 5mL(s). Cast substrate dependence of the film which can be created when change the substrate which carries out the cast with agarose gel, glass, a mica, and PHEMA, the flow rate of high humidity air is made a part for 2.0L/, relative humidity is made 30% and temperature is made into 20 degrees C was evaluated. The gestalt observation result at this time is shown in drawing 4 . In drawing 4 , apertures are a minimum of 7 micrometers and a maximum of 14 micrometers.

[0031] In addition, in drawing 1 - drawing 4 , all the die length of a bar is 20 micrometers.

[0032] Example 6 Evaluation of a filtration efficiency (1)

It preceded evaluating the hemofiltration engine performance of the film the honeycomb's created structure, particle size conducted the passage experiment of a known polystyrene particle, and the class and transmission coefficient of a particle which are passed by changing an aperture were investigated. A result is shown in Table 1. When the film of 5.5-8.5 micrometers of apertures was used, although the particle with a diameter of 3 micrometers was passed, it was able to be shown clearly that a particle with a diameter of 10 micrometers or more is not passed at all for the first time.

[0033]

[Table 1]

ポリスチレン粒子の通過率 膜の孔径依存

孔径 [μm]	ポリスチレン粒子の通過率 [%]		
	粒径3μm	粒径10μm	粒径20μm
5.5	60.1	0.0	0.0
8.5	98.6	0.0	0.0
11.5	99.6	5.2	0.0
17.5	100	79.8	0.0
25.5	100	97.7	43.2

粒子濃度 3μm：1．6×10^{7}個/mL
10μm：1．1×10^{6}個/mL
20μm：1．8×10^{7}個/mL

A particle transmission coefficient is measured at a particle counter.
[0034] Example 7 Evaluation of a filtration efficiency (2)
The prehension experiment of the leucocyte in Homo sapiens whole blood was conducted using the film of the honeycomb's created structure. When the man whole blood which collected blood with heparin blood collecting tubing was made to filter using 5.2 micrometers of apertures, and the 9.8-micrometer film, the cast of the blood was carried out to the counting chamber and the number of leucocytes was measured, before filtration, it turned out that the white blood cell count which 4800 piece [/micro] L Existed is zero piece [/micro] L after filtration (Table 2). Moreover, hemolysis was not checked when the color of the supernatant liquid obtained by carrying out centrifugal separation of the filtered filtrate by 3000 rotations for 10 minutes was checked visually.
[0035]
[Table 2]

白血球の捕捉能

孔径[μm]	白血球の通過率
5.5	0%
8.5	0%

4800 white blood cell count/micro[before filtration] L [0036] Example 8 Evaluation of a filtration efficiency (3)
The prehension experiment of the erythrocyte in Homo sapiens whole blood was conducted using the film of the honeycomb's created structure. Using the film of 3.5-5.5 micrometers of apertures, three sheets were pierced in the diameter of 5mm, the laminating was respectively, carried out to it, and it put on the nitrocellulose membrane. What was used as the whole blood which diluted the same whole blood with the plasma obtained by carrying out centrifugal separation, and prepared the Homo sapiens whole blood of 40% of hematocrit values which collected blood with heparin blood collecting tubing to 2.5% of hematocrit values On the film which carried out the laminating, wear 5micro L points, carry out, leave it for 10 seconds, and the film which carried out the laminating after that is lifted. By the film whose aperture is 3.5 micrometers when it evaluates whether the color with a red erythrocyte remains, saw a red color, and it was not stopped to the plasma which it was filtered and was imprinted to the nitrocellulose membrane, but it has checked that the erythrocyte could be caught (Table 3).
[0037]
[Table 3]

赤血球の捕捉能

孔径[μm]	転写した濾液の赤色味	赤血球捕捉の判定
3.5	赤色なし	○
4.0	赤色	×
4.2	赤色	×
5.5	赤色	×
4.5	赤色	×
4.5	赤色	×

[0038]
[Effect of the Invention] By this invention, a blood serum or plasma, and a corpuscle are separated, and only a leucocyte can be caught further and it can dissociate from the whole blood of a minute amount.

[Translation done.]

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DESCRIPTION OF DRAWINGS

[Brief Description of the Drawings]
[Drawing 1] Solvent dependence of the aperture when creating the honeycomb's film is shown.
[Drawing 2] The amount dependence of casts of the aperture when creating the honeycomb's film is shown.
[Drawing 3] Concentration dependence of the aperture when creating the honeycomb's film is shown.
[Drawing 4] The substrate dependence of the aperture when creating the honeycomb's film which carries out the cast is shown.

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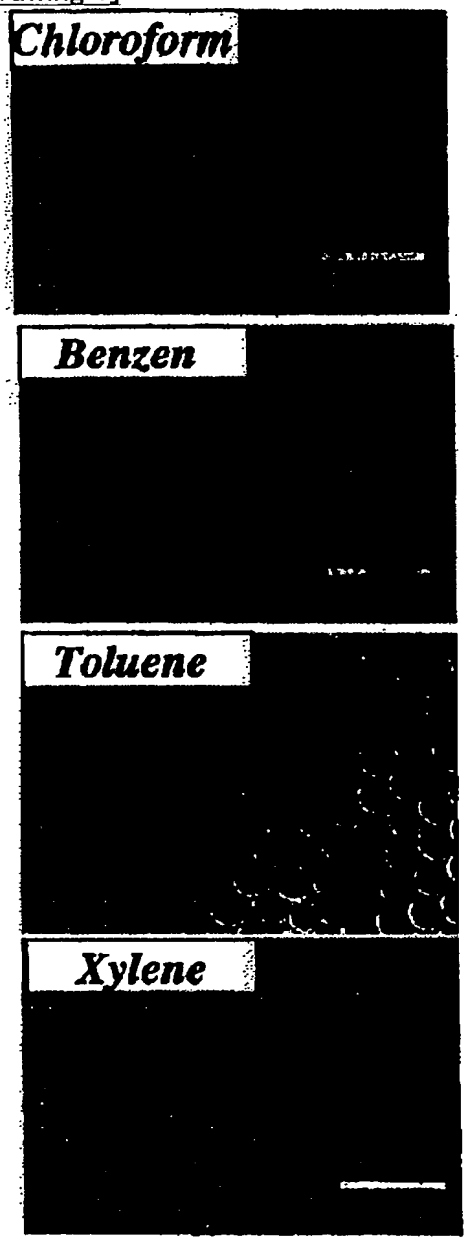
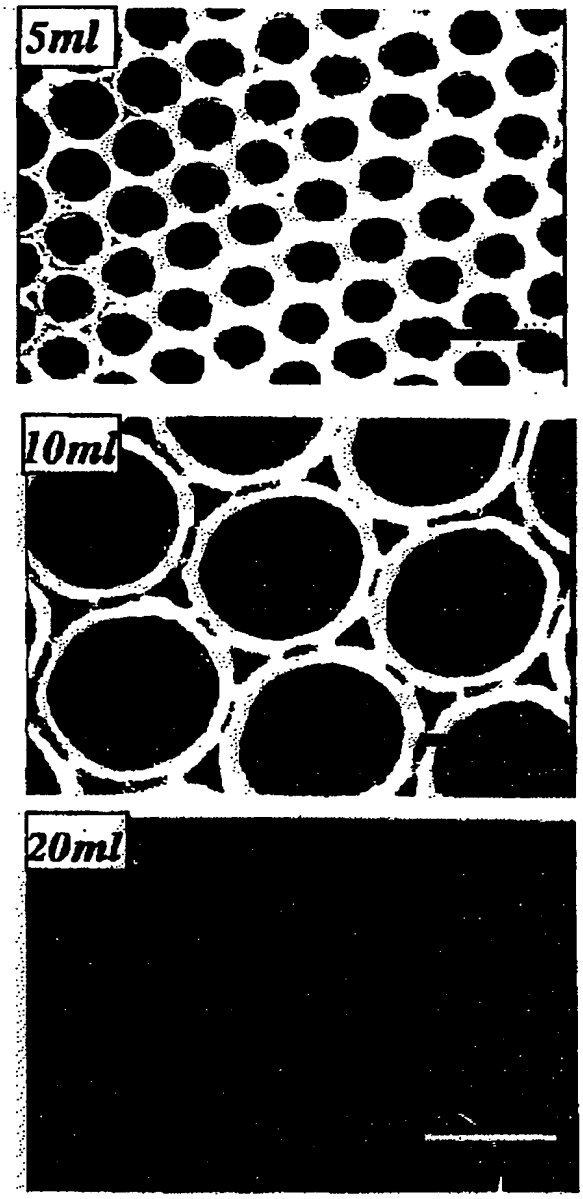
DRAWINGS

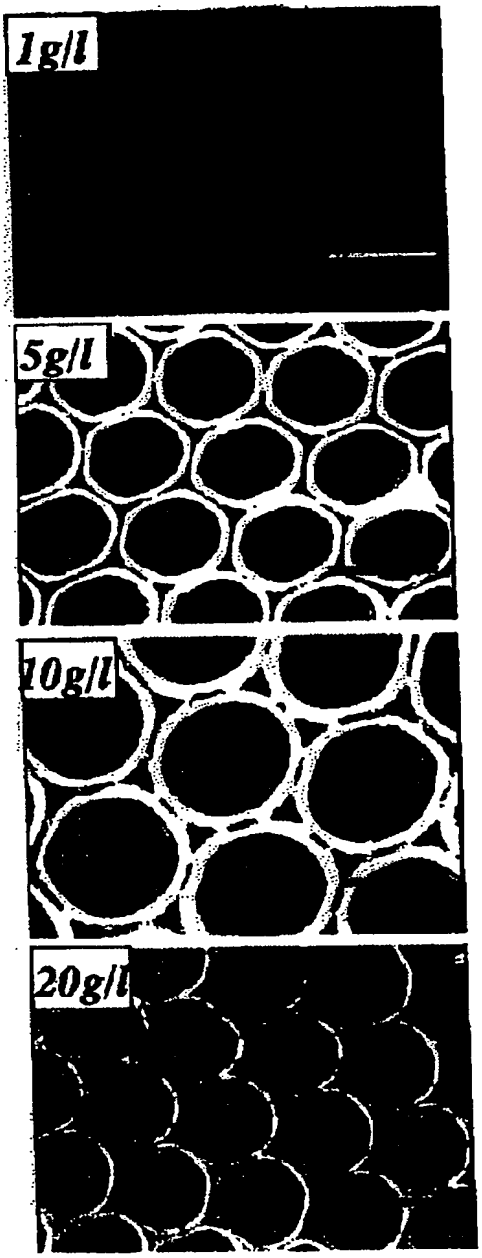
[Drawing 1]



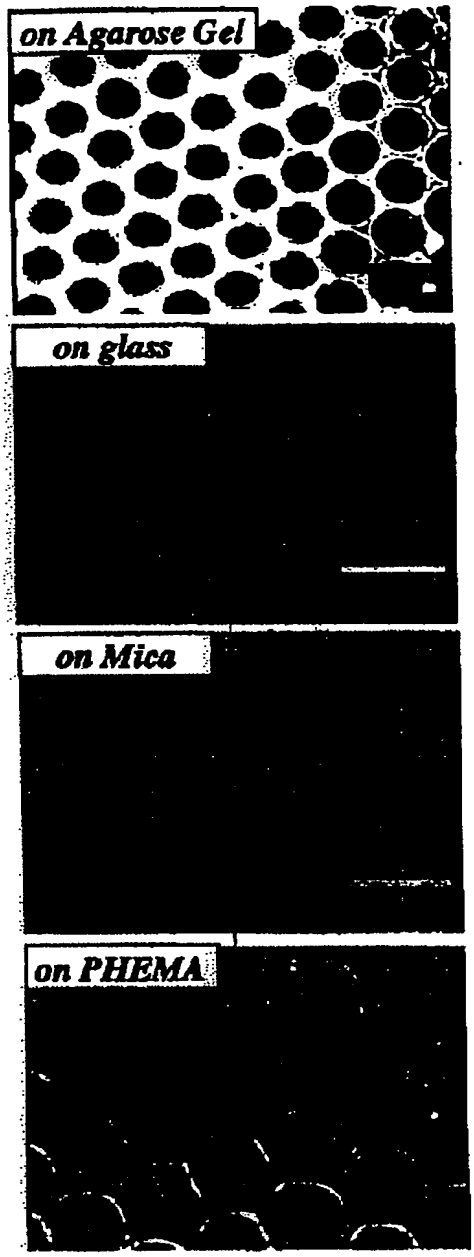
[Drawing 2]



[Drawing 3]



[Drawing 4]



[Translation done.]